PHENOLIC BINDING PEPTIDES

The present patent application claims priority of US patent application SN 60/417,210, filed 08 October 2002.

Background of the Invention

The present invention relates to novel binding peptides and to binding peptide conjugates, wherein the binding peptide is linked to an agent. In particular the peptides bind to tannin, polyphenolic and/or anthocyanin compounds and more particularly to tea and wine stains. The invention also concerns the use of the binding peptides for delivering agents to targeted tannin, polyphenolic and/or anthocyanin compounds that comprise tea and wine stains.

Binding peptides and proteins conjugated to a binding peptide have numerous uses in many varied applications. Some of these uses include applications in enzymatic compositions, particularly detergent compositions, in personal care applications, in food industry applications, in diagnostic applications and therapeutic applications.

For example oxidative-reductase (redox) enzymes capable of modifying the color associated with colored compounds could be used more effectively if conjugated to a peptide that targeted a particular compound. For example, a peptide that binds to a tannin, polyphenolic or anthocyanin compound as a target on a fabric or on a surface such as ceramic could deliver the redox enzyme more effectively to the specific target and result in more effective bleaching of the stain. This selective targeting of a tannin, polyphenolic or anthocyanin compound can provide a significant improvement in the cleaning performance of enzymatic compositions. In another example, a peptide that binds to a tannin, polyphenolic or anthocyanin compound on a surface such as skin, teeth or nails could deliver the redox enzyme more effectively to the specifically targeted pigmented areas which then may result in bleaching of the area .

Summary of the Invention

In a first aspect the invention concerns a peptide which binds to a compound selected from the group consisting of tannin, anthocyanin and phenolic compounds. In one preferred embodiment the binding peptide of the invention will bind to a tea or wine stain

and particularly to a tea or wine stain on a fabric or on a surface such as ceramic, glass, wood, paper, metal, plastic, skin, teeth, hair or nails.

In a second aspect the invention relates to a binding peptide comprising an amino acid sequence shown in any one of SEQ ID NOs. 1 – 316 and a binding peptide having at least 70% sequence identity thereto. In a further aspect the invention relates to a binding peptide consisting essentially of an amino acid sequence shown in any one of SEQ ID NOs. 1 – 316 and a binding peptide having at least 70% sequence identity thereto. In one embodiment the binding peptides of the invention further comprise a cysteine amino acid residue at the N and C terminus of a peptide as shown in any one of SEQ ID NOs. 1 - 316 or a binding peptide having at least 70% sequence identity thereto. In a another embodiment, the binding peptide is selected from the group consisting of KTPSPHG (SEQ ID NO. 1); PNTTRHS (SEQ ID NO. 2); LWTSPQL (SEQ ID NO. 8); TNNTSPT (SEQ ID NO. 24); SPTSTNS (SEQ ID NO. 43); TTTTPFA (SEQ ID NO. 77); SWNTSPL (SEQ ID NO. 80); QAVKASHATMYL (SEQ ID NO. 97); SYDLIPPRSGLA (SEQ ID NO. 104); DPNTTSH (SEQ ID NO.118); KASHLVP (SEQ ID NO: 132); LPTSTLT (SEQ ID NO. 139); QNQKSTT (SEQ ID NO. 158); SIIPPRQ (SEQ ID NO. 168); SNKPLSPNDLR (SEQ ID NO. 193) and peptides having at least 75% amino acid sequence identity thereto.

In a third aspect the invention concerns a binding peptide having a repeatable motif selected from the group consisting of LPL (SEQ ID NOs. 120, 123, 115 and 250); FAT (SEQ ID NOs. 125, 227 and 235); STT (SEQ ID NOs. 90, 158, 230 and 310); HSP (SEQ ID NOs. 18, 252 and 307); TNK (SEQ ID NOs. 40, 259 and 287); SPL (SEQ ID NOs. 53, 80, 152, 229, 232 and 292); THS (SEQ ID NOs. 62, 209 and 290); TSP (SEQ ID NOs. 8, 24, 80, 223 and 291); SPT (SEQ ID NOs. 24, 43 and 266); AQT (SEQ ID NOs. 59, 134 and 205); NSS (SEQ ID NOs. 31, 86, 213, 227 and 278); PAL (SEQ ID NOs. 109, 224 and 256); SGL (SEQ ID NOs. 104, 284 and 298); and TQT (SEQ ID NOs. 105, 281 and 287) and a binding peptide having at least 75% amino acid sequence identity thereto.

In a fourth aspect the invention concerns a binding peptide conjugate which comprises a binding peptide of the invention linked to an agent. In one embodiment the agent is a protein. In a preferred embodiment the protein is an enzyme and particularly an enzyme that catalyzes an oxidation-reduction reaction. In a particularly preferred embodiment the enzyme is selected from the group consisting of laccases, phenol oxidases, catalases, bilirubin oxidases, glucose oxidases and peroxidases. In one embodiment the binding peptide is covalently linked to said agent and in another embodiment the binding peptide and said agent are separated by a linker.

In a fifth aspect the invention relates to an enzymatic composition which comprises a binding peptide of the invention, an enzyme, and one or more surfactants. In one embodiment the composition is a detergent composition. In a second embodiment the enzymatic composition comprises a) a binding peptide conjugate which includes a binding peptide of the invention linked to an agent, wherein the agent is an enzyme and b) one or more surfactants. In a third embodiment the invention relates to a method for modifying a tea or wine stain on a fabric or a surface comprising contacting the fabric or surface having a tea or wine stain thereon with the enzymatic composition. Preferably the surface is ceramic, skin or teeth. Modification may include either removing the tea or wine stain or enhancing the tea or wine stain.

In a sixth aspect the invention relates to a method for delivering an agent to a target which comprises conjugating an agent with a binding peptide of the invention to form a binding peptide conjugate and exposing a target to the binding peptide conjugated, wherein the binding peptide conjugate binds to said target. In one embodiment the target is a tea or wine stain. In another embodiment the agent is an enzyme. In another embodiment the target is a tea or wine stain.

In a seventh aspect the invention relates to polynucleotide sequences encoding a binding peptide or a binding peptide conjugate according to the invention and to vectors and host cells comprising said polynucleotide sequences.

Brief Description of the Drawings

Figures 1A - 1B illustrate the amino acid sequences of peptides represented by SEQ ID NOs. 1 - 111 that bind to tea stains on cotton. A peptide string having an amino acid residue designated as X in a specific position indicates that the amino acid residue is not known and may be any L-amino acid.

Figures 2A - 2B illustrate the amino acid sequences of peptides represented by SEQ ID NOs. 112 - 201 that bind to tea stains on ceramic. A peptide string having an amino acid residue designated as X in a specific position indicates that the amino acid residue is not known and may be any L-amino acid.

Figures 3A, 3B and 3C illustrate the amino acid sequences of peptides represented by SEQ ID NOs. (202 - 316) that bind to wine stains on cotton. A peptide string having an amino acid residue designated as X in a specific position indicates that the amino acid residue is not known and may be any L-amino acid.

Figure 4 illustrates the preferential binding of phage bound peptides (PNTTRHS (SEQ ID NO. 2); LWTSPQL (SEQ ID NO. 8); TNNTSPT (SEQ ID NO. 24); SYGPMTN

(SEQ ID NO. 65); LHQNQKS (SEQ ID NO. 68); and SWNTSPL (SEQ ID NO. 80)) to tea stains on cotton swatches (■) compared to binding on non-stained cotton swatches (□). WT is a control, a phage without a binding peptide insert.

Detailed Description of the Invention

General Terms

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. For the purpose of the present invention, the following terms are used to describe the invention herein.

The term "peptide" refers to an oligomer in which the monomer units are amino acids (typically, but not limited to L-amino acids) linked by an amide bond. Peptides may be two or more amino acids in length. Peptides that are greater than 100 amino acids in length are generally referred to as polypeptides. However, the terms, peptide, polypeptide and protein may be used interchangeably. Standard abbreviations for amino acids are used herein and reference is made to Singleton *et al.*, (1987) DICTIONARY OF MICROBIOLOGY AND MOLECULAR BIOLOGY, 2ND ED. page 35.

The term "isolated" as used herein refers to a nucleic acid or amino acid sequence that is removed from at least one component with which it is naturally associated.

"Percent sequence identity" with respect to peptide or polynucleotide sequences refers to the percentage of residues that are identical in the two sequences. Thus 95% amino acid sequence identity means that 95% of the amino acids in the sequences are identical. Percent identity can be determined by direct comparison of the sequence information provided between two sequences and can be determined by various commercially available computer programs such as BESTFIT, FASTA, DNASTAR, TFASTA and BLAST.

A "binding peptide" according to the invention is a peptide that binds to a target with a binding affinity of at least about 10^{-2} M, at least about 10^{-3} M, at least about 10^{-4} M, at least about 10^{-5} M and preferably between about 10^{-2} M to 10^{-15} M, between about 10^{-2} M to 10^{-10} M and between about 10^{-2} M to 10^{-9} M.

The binding affinity of a peptide for its target or the binding affinity of a binding peptide conjugate for its target may be described by the dissociation constant (K_D). K_D is defined by k_{off}/k_{on}. The k_{off} value defines the rate at which a bound-target complex breaks apart or separates. This term is sometimes referred to in the art as the kinetic stability of the peptide-target complex or the ratio of any other measurable quantity that reflects the ratio

of binding affinity such as an enzyme-linked immunosorbent assay (ELISA) signal. K_{on} describes the rate at which the target and the binding peptide (or binding peptide conjugate) combine to form a bound-target complex. In one aspect, the k_{off} value for the bound-target complex will be less that about 10^{-2} sec^{-1}, less that about 10^{-3} sec^{-1}, less than about 10^{-4} sec^{-1} and also less than about 10^{-5} sec^{-1}.

The term "conjugation" as used herein means an agent is chemically linked or joined directly or indirectly to a terminus of a binding peptide. The phrases "binding peptide conjugate" and "conjugated agent" are used interchangeably herein. A binding peptide conjugate or a conjugated agent may be considered a fusion protein. A fusion protein refers to a protein that comprises two separate and distinct regions that may or may not originate from the same protein.

An "agent" is any molecule or compound that is capable of being conjugated with a binding peptide of the invention and preferably capable of be chaperoned or delivered to a target. Agents according to the invention comprise a broad class of compounds including but not limited to proteins, carbohydrates, lipids, chemicals, such as dyes, bleaching compounds and fluorescent compounds, and ions, such as salts.

Selectivity is defined herein as enhanced binding of a binding peptide to a target compared to the binding of the peptide to a non-target. Selectivity may also be defined as the enhanced binding of a conjugated agent to a target compared to the binding of a non-conjugated agent to the same target. Selectivity may be in the range of about 1.25 : 1 to 25 : 1; about 1.5 : 1 to 15 : 1; about 1.5 : 1 to 10 : 1; and about 1.5 : 1 to 5 : 1. Preferably the selectively is at least 4 :1, 3 : 1 or 2 : 1 for either a) the binding of a binding peptide to a target compared to the binding of the peptide to a non-target or b) the binding of a conjugated agent to a target compared to the binding of the non-conjugated agent to the same target.

Preferred targets of a binding peptide or a binding peptide conjugate of the invention are tannin, phenolic or anthocyanin compounds. Particularly tannin, phenolic or anthocyanin compounds found in tea or wine, and particularly a tea and/or wine stain. However, the target compounds may be found on a material, surface or solution.

A stain is defined herein as a colored compound which undergoes a redox chemical reaction when exposed to certain classes of enzymes, for example phenol oxidizing enzymes such as laccases. A coloured compound is a substance that adds colour to a textile or to substances which result in the visual appearances of stains. Targeted classes of coloured substances which may appear as a stain include the following;

a) porphyrin derived structures, such as heme in blood stain or chlorophyll in plants;

b) tannins and polyphenols (see P. Ribéreau-Gayon, Plant Phenolics, Ed. Oliver & Boyd, Edinburgh, 1972, pp.169-198) which occur in tea, wine, coffee, chocolate, cola, banana and peach stains;

c) carotenoids and carotenoid derivatives, which are the red, orange and yellow pigments occurring in fruits and vegetables such as tomato, mango, carrots, paprika and leafy green vegetables. Commonly known carotenoids include alpha and beta-carotene, lycopene, lutein, zeaxanthin, and cryptoxantin. These compounds include the oxygenated carotenoids, xanthophylls. Reference is made to G.E. Bartley et al., The Plant Cell (1995), Vol. 7, 1027-1038, Biochemical Nomenclature and Related Documents, 2nd Ed. Portland Press (1992), pages 226 – 238, and Pure Appl. Chem, (1974) 41:407 - 431);

d) anthocyanins, the highly coloured molecules which occur in many fruits and flowers, such as red grapes, cranberries, blueberries and cherries and red wine (P. Ribéreau-Gayon, Plant Phenolics, Ed. Oliver & Boyd, Edinburgh, 1972, 135-169); and

e) Maillard reaction products, the yellow/brown coloured substances which appear upon heating of mixtures of carbohydrate molecules in the presence of protein/peptide structures, such as found in cooking oil.

A coloured compound may also be a dye that may be incorporated into a fiber by chemical reaction, adsorption or dispersion. Examples include direct Blue dyes, acid Blue dyes, reactive Blue dyes, and reactive Black dyes.

A stain may occur on a fabric or other surface material. Nonlimiting examples of fabric include, cotton, wool, silk, polyester, rayon, linen, nylon and blends thereof. Nonlimiting examples of a surface material include, ceramic, glass, wood, paper, metal, plastic, stainless steel, teeth, bone, nails, skin and hair.

The phrase "modify the colour associated with a coloured compound" means that the coloured compound is changed through oxidation-reduction, either directly or indirectly, such that the colour appears modified i.e. the colour visually appears to be increased; decreased; changed from one color to another, such as from blue to red; decoloured; bleached; or removed; particularly bleached.

As used in the specification and claims, the singular "a", "an" and "the" include the plural references unless the context clearly dictates otherwise. For example, the term a host cell may include a plurality of host cells.

The following references describe the general techniques employed herein: Sambrook et al (1989) MOLECULAR CLONING: A LABORATORY MANUAL, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, NY; and Ausubel et al. (1987) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Greene-Publishing & Wiley Interscience NY (Supplemented through 1999). The contents of all references, patents and published

patent applications cited throughout this application are hereby incorporated by reference in their entirety.

B. Binding Peptides

The binding peptides of the invention may be obtained and identified using methods well known in the art. These methods may include the use of random peptide libraries, synthetic peptide libraries, peptide loop libraries, antibody libraries and protein libraries. Many of these library collections are commercially available. Screening techniques may include yeast display, ribosome display, biopanning and acid elution. Once a library is screened, the peptides that bind to a specific target may be identified by various well-known means in the art including but not limited to acid elution, polymerase chain reaction (PCR), sequencing, and the like. These techniques are described in various references such as Cwirla et al., (1990) *Proc. Natl. Acad. Sci. USA* 87:6378; Parmley et al., (1988) *Gene* 73:305; Balass et al., (1996) *Anal. Biochem.*, 243:264; Huls et al., (1996) *Nature Biotechnol.*, 7:276 and WO 01/79479).

A typical method for selecting binding peptides of the invention involves removing from a library those peptides that bind non-specifically to a material and then incubating the remaining members of the library with a stained material containing the target substrate.

Once selected a binding peptide may be identified, amplified or produced in bulk by any one of a number of standard techniques. For example the peptide may be produced recombinantly using genetic engineering or the peptide may be chemically synthesized. Preferably the binding peptides of the invention are between 4 and 50 amino acids in length, also between 4 - 25 amino acids in length, between 4 - 20 amino acids in length and between 6 -15 amino acids in length.

The binding peptides according to the invention include the peptides listed in figures 1A -1B (SEQ ID NOs. 1 - 111), figures 2A - 2B (SEQ ID NOs. 112 - 201); and figures 3A, 3B and 3C (SEQ ID NOs. 202 - 316. These peptides bind to molecules found in tea and/or wine.

The invention further includes binding peptides having at least 60% but less than 100% amino acid sequence identity to a binding peptide listed in figures 1A, 1B, 2A, 2B, 3A, 3B or 3C (SEQ ID NOs. 1 - 316). For example at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 93%, at least 95%, at least 97%, at least 99% amino acid sequence identity. A peptide having at least 60% sequence identity to a binding peptide listed in figures 1A, 1B, 2A, 2B, 3A, 3B or 3C will also have a binding affinity for the same target in the range of 10^{-2}M to 10^{-15}M, generally at least about 10^{-2}M, at least about 10^{-3}M, at least about 10^{-4}M and at least about 10^{-5}M. In one embodiment a

binding peptide according to the invention will have no more than 2 amino acid residues that differ from a binding 7-mer peptide which is listed in figures 1A, 1B, 2A, 2B, 3A, 3B or 3C. In another embodiment, a binding peptide according to the invention will have no more than 3 amino acid residues that differ from a binding 12-mer peptide which is listed in figures 1A, 1B, 2A, 2B, 3A, 3B or 3C.

In one embodiment, preferred binding peptides of figures 1A – 1B are: KTPSPHG (SEQ ID NO. 1); PNTTRHS (SEQ ID NO. 2); KTPSSME (SEQ ID NO. 5); LWTSPQL (SEQ ID NO. 8); SLNNTNT (SEQ ID NO. 11); QKHSPGH (SEQ ID NO. 18); TNNTSPT (SEQ ID NO. 24); QTQPPGS (SEQ ID NO. 25); TMAPAKN (SEQ ID NO. 36); SHLDKRL (SEQ ID NO. 37); TTTNKPL (SEQ ID NO. 40); SPTSTNS (SEQ ID NO. 43); PGSNATQ (SEQ ID NO. 44); SQDTPMY (SEQ ID NO. 45); TDPSMMN (SEQ ID NO. 46); GQADRLQ (SEQ ID NO. 47); TPQRLLT (SEQ ID NO. 48); SQMSPLH (SEQ ID NO. 53); TQNPTHS (SEQ ID NO. 62); HGSSAHP (SEQ ID NO. 64); TTAAPQM (SEQ ID NO. 70); SSNLPFA (SEQ ID NO. 71); TTTTPFA (SEQ ID NO. 77); SWNTSPL (SEQ ID NO. 80); PSPPTNQ (SEQ ID NO. 82); PLTSTQP (SEQ ID NO. 85); HVSDLAG (SEQ ID NO. 87); TLSRTTA (SEQ ID NO. 88); HLRSTTD (SEQ ID NO. 90); SPMQPRL (SEQ ID NO. 93); FTANLRA (SEQ ID NO. 94); LFLPPTPPPEPL (SEQ ID NO. 96); QAVKASHATMYL (SEQ ID NO. 97); ETQPSAMGGSSL (SEQ ID NO. 99); STSWPPQPHLSP (SEQ ID NO. 102); SYDLIPPRSGLA (SEQ ID NO. 104); NTTQTLRHVSLA (SEQ ID NO. 105); TSGFDRALSPSL (SEQ ID NO. 107); SNSTMNALAPA (SEQ ID NO. 111) and peptides having at least 70% amino acid sequence identity thereto.

Particularly preferred binding peptides of figures 1A and 1B are PNTTRHS (SEQ ID NO. 2); LWTSPQL (SEQ ID NO. 8); TNNTSPT (SEQ ID NO. 24); and SWNTSPL (SEQ ID NO. 80) and peptides having at least 75% amino acid sequence identity thereto.

In another embodiment, preferred binding peptides of figures 2A – 2B are: ALGXIPXTAHQW (SEQ ID NO. 114); ARSIQPF (SEQ ID NO. 115); ATVILTD (SEQ ID NO. 116); DPNTTSH (SEQ ID NO. 118); FLPLLTL (SEQ ID NO. 120); FQLIPTG (SEQ ID NO. 121); GVPFATP (SEQ ID NO. 125); IPTTRQT (SEQ ID NO. 131); KASHLVP (SEQ ID NO. 132); KDPSWPSQAQTP (SEQ ID NO. 134); LPTSTLT (SEQ ID NO. 139); PPSPLTP (SEQ ID NO. 152); PTLAGAS (SEQ ID NO. 154); QDTAPLT (SEQ ID NO. 157); QNQKSTT (SEQ ID NO. 158); QPGHLDI (SEQ ID NO. 159); LSLPMQ (SEQ ID NO. 164); SIIPPRQ (SEQ ID NO. 168); SSLLPRS (SEQ ID NO. 174); TAPLISI (SEQ ID NO. 177); TKTTWQT (SEQ ID NO. 180); TLFYTKX (SEQ ID NO. 181); TQRLTTH (SEQ ID NO. 182); TSLVPDK (SEQ ID NO. 184); WQLARPK (SEQ ID NO. 191); WQTXLTD (SEQ ID NO. 192); WSNKPLSPNDLR (SEQ ID NO. 193); YTKTSQY (SEQ ID NO. 201); and peptides having at least 70% amino acid sequence identity thereto.

In another embodiment preferred binding peptides of figures 1A, 1B, 2A and 2B include KTPSPHG (SEQ ID NO. 1); PNTTRHS (SEQ ID NO. 2); LWTSPQL (SEQ ID NO. 8); TNNTSPT (SEQ ID NO. 24); SPTSTNS (SEQ ID NO. 43); TTTTPFA (SEQ ID NO. 77); SWNTSPL (SEQ ID NO. 80); QAVKASHATMYL (SEQ ID NO. 97); SYDLIPPRSGLA (SEQ ID NO. 104); DPNTTSH (SEQ ID NO.118); KASHLVP (SEQ ID NO: 132); LPTSTLT (SEQ ID NO. 139); QNQKSTT (SEQ ID NO. 158); SIIPPRQ (SEQ ID NO. 168); WSNKPLSPNDLR (SEQ ID NO. 193) and peptides having at least 75% amino acid sequence identity thereto.

In another embodiment, preferred binding peptides of figures 3A, 3B and 3C are: QYHGPLP (SEQ ID NO. 203); TGNSSQQ (SEQ ID NO. 213); LPLQPLMPPLNQ (SEQ ID NO. 225); NSSPFATMPNAL (SEQ ID NO. 227); NVNNHIH (SEQ ID NO. 247); ADRLRPT (SEQ ID NO. 251); HSPQMQS (SEQ ID NO. 252); SPALVNS (SEQ ID NO. 256); TNKIPPL (SEQ ID NO. 259); TNPNHIM (SEQ ID NO. 260); QPLKTKQ (SEQ ID NO. 262); TKSPTAI (SEQ ID NO. 266); KSPEYPF (SEQ ID NO. 270); TTQTNKD (SEQ ID NO. 287); PATNPNH (SEQ ID NO. 289); SPLYHDR (SEQ ID NO. 292); NAFESLF (SEQ ID NO. 296); DPQANLT (SEQ ID NO. 299); RQANLTQ (SEQ ID NO. 300); LDQHSMK, (SEQ ID NO. 301); PSTTKHG (SEQ ID NO. 310); and peptides having at least 70% amino acid sequence identity thereto.

In a further embodiment, the binding peptides according to the invention may include cysteine residues on each end of the peptide. These binding peptides are more specifically referred to herein as binding peptide C-C derivatives. The cysteine residues form a disulfide bridge, making the peptide form a loop on the surface of the phage. Thus, if the binding peptide is used as an internal replacement or insert for protein loops or turns, the binding peptide may be used in the C-C derivative form or the non C-C derivative form. Particularly preferred C-C derivative peptides are those comprising 7 amino acids. In one aspect preferred C-C derivatives are the preferred 7-mers disclosed in figures 1A - 1B; figures 2A - 2B and figures 3A, 3B and 3C as designated above.

Additionally, a linker (L) molecule (also sometimes referred to as a spacer moiety in the prior art) may be added to either end of a binding peptide (P), for example, L-P or P-L. The linker molecule may enhance the binding of the peptide to its target. A linker molecule may be any carbon containing compound, such as a short peptide, for example, the amino acid triad GGH or GGHGG; a carbon chain, for example, $(CH_2)_n$ wherein n equals 1 to 10; a polymer, for example PEG $(CH_2\text{-}O)_n$ wherein n equals 2 - 20; a sugar; a lipid or the like.

As stated above, the linker molecule may be attached to the binding peptide alone or the linker molecule may be part of the binding peptide conjugate. For example, when the linker (L) is placed between the binding peptide (P) and the agent (A), (A-L-P) or when the

linker is attached to the peptide at the non-conjugated end, (A-P-L). A linker molecule may be attached to any of the binding peptides represented as SEQ ID NOs. 1 - 316 of figures 1A, 1B, 2A, 2B, 3A, 3B and 3C.

Repeatable motifs have been observed in a number of the binding peptides listed in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C. Repeatable motifs include at least three consecutive amino acid residues in a peptide string and may include four, five or six consecutive amino acid residues that are found in at least two of the binding peptides listed in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C.

Preferred repeatable motifs which are included in binding peptides listed in figures 1A and 1B, which bind to a tea stain are: INAQ, KTPS, NSSS, NTSP, SNAT, GSS, HQT, PGS, SSS, TPQ, TQP, TSP, TTA, TTT, APA, HQG, HPS, HVS, KPL, LNN, LPF, SNS, SPL, SPM, SPT, SRL, LSP, LSR, MMN, MYL, NAQ, NNT, NPT, NTT, PAK, PFA, PLH, PPP, PPT, PQM, PSL, PSP, PTH, QKH, RLQ, SLA, SNA, TQK, TQM, TQN, TSG, TST, STM, STR, TAA, TDP, TMA, TTP, TTQ, VTT, AND QNQ.

Particularly preferred repeatable motifs and peptides which include these motifs of figures 1A and 1B are INAQ, (SEQ ID NOs. 59 and 84); KTPS, (SEQ ID NOs. 1 and 5); NSSS, (SEQ ID NOs. 31 and 86); NTSP, (SEQ ID NOs. 24 and 80); SNAT, (SEQ ID NOs. 32 and 44); GSS, (SEQ ID NOs. 64, 99 and 100); HQT, (SEQ ID NOs. 7, 15 and 108); PGS, (SEQ ID NOs. 25, 44 and 100); TPQ, (SEQ ID NOs. 14, 48 and 66); TQP, (SEQ ID NOs. 25, 85 and 99); TSP, (SEQ ID NOs. 8, 24 and 80) and TTA, (SEQ ID NOs. 28, 70 and 88).

Preferred repeatable motifs which are included in binding peptides listed in figures 2A and 2B which bind to a tea stain are: ATP, APL, HPP, IPT, ISI, KTSQ, LPR, LPM, LPL, LPT, LST, LTD, LTP, LVP, LSP, PLI, PPR, PAP, PTL, PLT, SLV, SWP, TSQ, TAPL, TLF, TLT, TKT, WQT, and YTK.

Particularly preferred repeatable motifs and peptides which include these motifs of figures 2A and 2B are LTP, (SEQ ID NOs. 142, 152 and 163) and LSP, (SEQ ID NOs. 137, 176 and 193).

Preferred repeatable motifs which are included in binding peptides listed in figures 3A, 3B and 3C which bind to a wine stain are: QANLT; TNPNH; ANLT; NPNH; QANL; TNPN; PPL; SPL; AAT; ANL; DRL; ELP; FAT; GLS; HAM; HGP; HQA; HSP; KSP; KTK; LHD; LPL; LPP; LYH; MPN; MQS; NAF; NHI; NLT; NMN; NPN; NTL; NVN; NSS; PAL; PAT; PHP; PLM; PLN; PLP; PNH; PTA; PYT; QPL; QTN; RLH; RSA; SGL; SHS; SLF; SPQ; SRS; STP; STS; STT; TAE; TFA; TGN; THS; TKH; TNK; TNP; TPP; TPR; TQT; TRS; TSP; TTI; VNS; WNA; and YPF.

Particularly preferred repeatable motifs and peptides which include these motifs of figures 3A, 3B and 3C are QANLT (SEQ ID NOs. 299 and 300); TNPNH (SEQ ID NOs. 260 and 289); ANLT (SEQ ID NOs. 300 and 299); NPNH (SEQ ID NOs. 260 and 289); QANL (SEQ ID NOs. 299 and 300); TNPN (SEQ ID NOs. 260 and 289); NSS (SEQ ID NOs, 213, 227 and 278); PPL (SEQ ID NOs. 225, 229 and 259) and SPL (SEQ ID NOs. 229, 232 and 292).

Preferred repeatable motifs for peptides that bind to compounds in wine and tea and binding peptides including these repeatable motifs are the following: LPL (SEQ ID NOs. 120, 123, 115 and 250); FAT (SEQ ID NOs. 125, 227 and 235); STT (SEQ ID NOs. 90, 158, 230 and 310); HSP (SEQ ID NOs. 18, 252 and 307); TNK (SEQ ID NOs. 40, 259 and 287); SPL (SEQ ID NOs. 53, 80, 152, 229, 232 and 292); THS (SEQ ID NOs. 62, 209 and 290); TSP (SEQ ID NOs. 8, 24, 80, 223 and 291); SPT (SEQ ID NOs. 24, 43 and 266); AQT (SEQ ID NOs. 59, 134 and 205); NSS (SEQ ID NOs. 31, 86, 213, 227 and 278); PAL (SEQ ID NOs. 109, 224 and 256); SGL (SEQ ID NOs. 104, 284 and 298); and TQT (SEQ ID NOs. 105, 281 and 287).

The repeatable motif may also include a cysteine residue at the beginning and/or end of the motif, non-limiting examples include (C)SPM, (C)SPL, (C)KTPS, (C)TTT, TTA(C) and the like.

In general, the repeatable motifs may occur alone in a binding peptide, as multiple motifs in the same binding peptide, in sequential order, or overlapping one another. For example the binding peptide KTPSPHG (SEQ ID NO: 1) includes the repeatable motif KTPS. The binding peptide LGTPQQT (SEQ ID NO: 14) includes the repeatable motif TPQ. The binding peptides RQANLTQ (SEQ ID NO. 300) and DPQANLT (SEQ ID NO. 299) include the repeatable motif QANLT. The binding peptides TTAAPQM (SEQ ID NO. 70) and ETQPSAMGGSSL (SEQ ID NO. 99) include two repeatable motifs, in the same sequence. The binding peptide LPLQPLMPPLNQ (SEQ ID NO. 225) includes two repeatable motifs LPL and QPL in sequential order.

Peptides, other than the binding peptides illustrated in figures 1A,1B, 2A, 2B, 3A, 3B and 3C, which have a repeatable motif as disclosed herein above are referred to herein as "homologous motif binding peptides". Homologous motif binding peptides will include 6 – 25 amino acid residues, preferably 6 - 15 amino acid residues and more preferably 6 to 12 amino acid residues. Further a homologous motif binding peptide will bind to a target with a binding affinity similar to or greater than the binding affinity to the same target as a binding peptide of figure 1A, 1B, 2A, 2B, 3A, 3B or 3C having the same repeatable motif. Preferably the target will be a tannin, phenolic or anthocyanin compound, most preferably a

tea or wine stain, and the binding affinity will be at least about 10^{-2}M, at least about 10^{-3}M, at least about 10^{-4}M, at least about 10^{-6}M and generally between about 10^{-2}M and 10^{-9}M.

A homologous motif binding peptide will include not only a repeatable motif as defined herein, but also will have between 20% and 95% amino acid sequence identity with a sequence illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C having the same repeatable motif, that is at least 25% sequence identity, at least 30% sequence identity, at least 40% sequence, at least 50% sequence identity, at least 60% sequence identity, at least 70% sequence identity, at least 80% sequence identity, at least 85% sequence identity, at least 90% sequence identity or at least 95% sequence identity to a binding peptide illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C which includes the same repeatable motif. Preferably if the homologous motif binding peptide is a 7 amino acid residue peptide, the homologous motif binding peptide will have at least 30% sequence identity with a binding peptide illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C having the same repeatable motif when the peptides are aligned with no gaps. If the homologous motif binding peptide is a 12 amino acid residue peptide, the peptide will have at least 25% sequence identity with a binding peptide illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C having the same repeatable motif when the peptides are aligned with no gaps.

C. Polynucleotides encoding the binding peptides

The present invention encompasses polynucleotides which encode binding peptides according to the invention. Specifically polynucleotides include nucleic acid sequences encoding peptides illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B and 3C (SEQ ID NOs. 1 - 316) and their C-C derivatives. Additionally, polynucleotides of the invention will encode binding peptides having at least 70% amino acid sequence identity to a peptide illustrated in figures 1A, 1B, 2A, 2B, 3A, 3B or 3C (SEQ ID NOs. 1 - 316), their C-C derivatives and homologous motif binding peptides. As will be understood by the skilled artisan, due to the degeneracy of the genetic code, a variety of polynucleotides can encode a binding peptide of the invention. The present invention encompasses all such polynucleotides. A polynucleotide which encodes a binding peptide of the invention may be obtained by standard procedures known in the art, for example, by chemical synthesis, by PCR and by direct isolation and amplification.

D. Conjugation of binding peptides to an agent.

In one embodiment, a binding peptide conjugate is formed wherein a binding peptide according to the invention is linked with an agent. While agents may include

proteins, carbohydrates, lipids and ions as described above, various preferred agents are listed below.

In one aspect an agent may be a protein. The protein may be an enzyme, a hormone, a growth factor, a cytokine, an antibody, and an anti-astringent protein or other protein.

Enzymes include but are not limited to amylolytic enzymes, proteolytic enzymes, cellulolytic enzymes, redox enzymes, transferases and cell wall degrading enzymes. Examples of these enzymes include, but are not limited to, amylases, proteases, xylanases, lipases, laccases, phenol oxidases, oxidases, such as glucose oxidases and galactoses, oxidases, peroxidases, cutinases, catalases, cellulases, hemicellulases, esterases, pectinases, glycosidases, isomerases, transferases, galactosidases, pullulanases, epimerases, phytases, hydroxylases, epoxydases, alkyltransferases and chitinases.

Hormones include, but are not limited to, follicle-stimulating hormone, luteinizing hormone, corticotropin-releasing factor, somatostatin, gonadotropin hormone, vasopressin, oxytocin, erthropoietin, insulin and the like.

Growth factors are proteins that bind to receptors on the cell surface, with the primary result of activating cellular proliferation and/or differentiation. Growth factors include, but are not limited to, platelet-derived growth factors, epidermal growth factor, nerve growth factor, fibroblast growth factors, insulin-like growth factors, transforming growth factors and the like.

Cytokines are a unique family of growth factors. Secreted primarily from leukocytes, cytokines stimulate both the humoral and cellular immune responses, as well as the activation of phagocytic cells. Cytokines include, but are not limited to, colony stimulating factors, the interleukins (IL-1 (α and β), IL-2 through IL-13) and the interferons (α, β and γ).

Antibodies include, but are not limited to, immunoglobulins from any species from which it is desirable to produce large quantities. It is especially preferred that the antibodies are human antibodies. Immunoglobulins may be from any class, i.e., G, A, M, E or D.

Anti-astringency compounds include proteins or carbohydrates that reduce the astringency of other compounds by either binding to them and/or precipitating them. Anti-astringency proteins include but are not limited to casein and albumin.

The agent may also be a vitamin, such as thiamin, riboflavin, niacin, pantothenic acid, pyridoxal, pyridoxamine, pyridoxine, biotin, cobalamin, folic acid, ascorbic acid, vitamin A, vitamin D, vitamin E and vitamin K.

Sweeteners which may be agents include carbohydrates and sugar alcohols such as, raw sugar, corn sweetener, corn syrup, dextrose, sucrose, granulated sugar, brown sugar, confectioner's sugar, honey, lactose, maltose, mannitol, sorbitol, aspartame.

An agent may be a bleaching compound, such as an oxygen bleaching agent or halogen bleaching agent. Non-limiting examples of oxygen bleaching agents include perborate, percarbonate, sulfate/hydrogen peroxide, and percarboxylic acid. Non-limiting examples of halogen bleaching agents include hypohalite and hypochlorite bleaching agents such as trichloro- isocyanuric acid, sodium and potassium dichloro-isocyanurate and N-chloro and N-bromo alkanesulphonamides. Ions such as salts for example potassium, calcium and bicarbonates may also be agents.

An agent may also be a dye such as a fluorescent dye. For example, fluorescein isothiocynate (FITC), rhodamine, phycoerytherin, phycocyanin, fluorescamine and green fluorescent protein (GFP). Fluorescent dyes are disclosed in British Patent Appl. No. 2094826.

Particularly preferred agents are proteins such as enzymes. In one embodiment preferred enzymes are oxidoreductase enzymes. These enzymes include dehydrogenases, reductases, oxidases, synthases, monooxygenases, isomerases, lipoxygenases, dioxygenases and hydroxylases. More specifically preferred oxidoreductase enzymes as agents include laccases (EC 1.10.3.2), phenol oxidases (EC 1.14.18.1), catalases (EC 1.11.1.6), bilirubin oxidases (EC 1.3.3.5), catechol oxidases (EC 1.10.3.1), peroxidases (EC 1.11.1.7), and glucose oxidases (EC 1.1.3.4). Other preferred enzymes include amylases, proteases, xylanases, lipases, transferases and cellulases.

Numerous references are available on suitable enzymes which may be linked with a binding peptide according to the invention to form a binding peptide conjugate. Proteins conjugated with a binding peptide of the invention may be recombinant proteins or naturally occurring proteins. Oxidoreductase enzymes, such as phenol oxidizing enzymes and particularly laccases, and polynucleotides encoding said enzymes which may be conjugated with a binding peptide of the invention are disclosed for example in WO 98/27197; WO 98/27198; WO 98/38286; WO 99/49020; WO 00/37654; WO 01/21809; U.S. Pat. No. 4,760,025; U.S. Pat. No. 5,770,419; U.S. Pat. No. 5,985,818; U.S. Pat. No. 6,060,442; and US Pat. No. 6,168,936. Proteases, such as subtilisins are disclosed in USP 6,197,567; USP 6,190,900; USP 6,110,884; EP 130756; EP 251446; EP 260105; EP525610; WO 87/04461 and WO 94/02618. Cellulases are disclosed in USP 5,989,899; USP 6,063,611; USP 6,268,328; USP 6,287,839 and USP 6,423,524. Amylases are disclosed in USP 6,440,716. Lipases are disclosed in USP 6,156,552; EP 407225; WO 95/06720; WO 95/22615; and WO 96/27002.

A binding peptide of the invention may act to deliver an agent to a target. The term deliver or delivering means to assist in the movement of the agent. In one embodiment the agent, particularly an enzyme, is delivered to a compound selected from tannin, polyphenolic or anthocyanin compounds and most particularly a tannin, polyphenolic or anthocyanin stain on a fabric or surface.

E. Making the binding peptide conjugate.

The binding peptide conjugate may be constructed by methods well known in the art including use of PCR. A binding peptide according to the invention may be inserted into an agent or attached to a terminus of the agent. When the agent is a protein a) the binding peptide may be inserted into the protein b) the binding peptide may replace an internal loop or turn, and/or c) the binding agent may be attached to the carbon or nitrogen terminus of the enzyme. In a preferred embodiment the agent is a protein (particularly an enzyme) and the binding peptide is linked to the carbon terminus of the agent. An agent may also be linked to a binding protein by chemical modification such as by an ester linkage or an amide linkage. Various methods of conjugating peptides to an agent are disclosed for example in USP 6,348,317; WO 02/57299; WO 02/55543; WO 02/26782; WO 00/48464; and WO 98/34956.

F. Expression systems, transformation and cultivation of host cells.

The present invention provides vectors, host cells, expression methods and systems for the production of the binding peptides and binding peptide conjugates in host microorganisms, such as bacteria, fungus and yeast.

Molecular biology techniques are disclosed in Sambrook et al., MOLECULAR BIOLOGY CLONING : A LABORATORY MANUAL, 2nd Ed (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. A polynucleotide encoding a binding peptide or a binding peptide conjugate is obtained and transformed into a host cell using appropriate vectors. A variety of vectors and transformation and expression cassettes suitable for the cloning, transformation and expression of proteins in fungus, yeast and bacteria are known by those of skill in the art.

Vectors will further include initiation control regions or promoters, which are useful to drive expression of the binding peptide or binding peptide conjugates in a host cell. Regulatory control elements are known to those skilled in the art. Virtually any promoter capable of driving the expression of the particular agent is suitable for the present invention. Once suitable cassettes are constructed they are used to transform a host cell.

Preferably a host cell is a microbial host cell, and preferably a bacteria, fungal or yeast host cell. In one embodiment the host cell is a gram positive bacteria, preferably a *Bacillus* species, such as *B. subtilis*. In another embodiment the host cell is a gram negative host cell, preferably an *Escherichia* species, such as *E. coli*. In other embodiments the host cell is fungal host cell, such as a filamentous fungus including a *Aspergillus* species, a *Trichoderma* species and a *Mucor* species. Particularly preferred are *T. reesei*, *A. niger* and *A. oryzae*.

One skilled in the art is well aware of methods of transforming host cells with polynucleotides encoding a protein of interest. General transformation procedures are taught in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY (vol. 1, edited by Ausubel et al., John Wiley & Sons, Inc. 1987, Chapter 9) and include calcium phosphate methods, transformation using PEG and electroporation. For *Aspergillus* and *Trichoderma*, PEG and calcium mediated protoplast transformation can be used (Finkelstein, DB 1992 TRANSFORMATION. IN BIOTECHNOLOGY OF FILAMENTOUS FUNGI. TECHNOLOGY AND PRODUCTS (eds. by Finkelstein & Bill) 113-156. Electroporation of protoplast is disclosed in Finkelestein, DB 1992 Transformation. In BIOTECHNOLOGY OF FILAMENTOUS FUNGI. TECHNOLOGY AND PRODUCTS (eds. by Finkelstein & Bill) 113-156. Microprojection bombardment on conidia is described in Fungaro et al. (1995) Transformation of Aspergillus nidulans by microprojection bombardment on intact conidia. *FEMS Microbiology Letters* 125 293-298. Agrobacterium mediated transformation is disclosed in Groot et al. (1998) Agrobacterium tumefaciens-mediated transformation of filamentous fungi. *Nature Biotechnology* 16 839-842. For transformation of Saccharomyces, lithium acetate mediated transformation and PEG and calcium mediated protoplast transformation as well as electroporation techniques are known by those of skill in the art.

Transformation of *Bacillus* is described, for example in Chang and Cohen (1979) *Mol. Gen Genet.* 168:111-115; Smith et al. (1986) *Appl. and Env. Microbiol.* 51:634; Mann et al. (1986) *Current Microbiol.* 13: 131-135. Also general reference is made to MOLECULAR BIOLOGICAL METHODS FOR *BACILLUS*. Eds. Hardwood and Cutting, John Wiley & Sons (1990).

A binding peptide or a binding peptide conjugate, particularly an enzyme conjugate wherein the enzyme is an oxidoreductase, a protease, an amylase, a xylanase, a lipase or a cellulase, may be produced by cultivation of a host cell which includes a polynucleotide encoding the binding peptide or enzyme conjugate, under aerobic conditions in nutrient media containing assimilable carbon and nitrogen together with other essential nutrient. These conditions are well known in the art.

for removing food stains on fabrics or removing food stains on surfaces such as ceramic and teeth.

Enzymatic compositions may also comprise additional components, such as for example, for formulation or as performance enhancers. For example, detergent compositions may comprise, in addition to the binding peptide conjugate, conventional detergent ingredients such as surfactants, builders and enzymes. Surfactants include nonionic, anionic and cationic surfactants (see EP-A-346995). Enzymes include for example, proteases, amylases, lipases, cutinases, cellulases and peroxidases (US Pat. No. 4,689,297). Other ingredients include enhancers, stabilizing agents, bactericides, optical brighteners and perfumes. The enzymatic compositions may take any suitable physical form, such as a powder, an aqueous or non-aqueous liquid, a paste or a gel. Reference is made to US Pat. No. 3,929,678; US Pat. No. 4,760,025; US Pat. No.5,011,681; WO 97/04079; WO 97/076202; WO 96/06930; WO 95/01426 and McCutheon's Detergents and Emulsifiers, North American Ed. (1986) Allured Publishing Co.

A binding peptide conjugate, particularly when the agent is an enzyme and more particularly when the agent is a redox enzyme such as a laccase, can act to modify the color associated with dyes or colored compounds in the presence or absence of enhancers depending upon the characteristics of the colored compound. If a compound is able to act as a direct substrate for the binding peptide conjugate, the phenol oxidizing enzyme will modify the color associated with a dye or colored compound in the absence of an enhancer, although an enhancer may still be preferred for optimum phenol oxidizing enzyme activity. For other colored compounds unable to act as a direct substrate for the binding peptide conjugate or not directly accessible to the conjugate, an enhancer may be required for optimum enzyme activity and modification of the color.

Enhancers are described in for example WO 95/01426, WO 96/06930, and WO 97/11217. Enhancers include but are not limited to phenothiazine-10-propionic acid (PTP), 10-methylphenothiazine (MPT), phenoxazine-10-propionic acid (PPO), 10-methylphenoxazine (MPO), 10-ethylphenothiazine-4-carboxylic acid (EPC) acetosyringone, syringaldehyde, methylsyringate, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate (ABTS), 2, 6 dimethoxyphenol (2,6-DMP), and guaiacol (2-methoxyphenol).

While enzymes and their use in detergent and cleaning compositions are well known, a main advantage of a binding peptide conjugate according to the invention is the delivery of an agent to a target and the enhanced binding of the conjugate to a target stain compared to the agent without the binding peptide.

Host cells that comprise a coding sequence for a binding peptide or binding peptide conjugate and express the binding peptide may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization and protein bioassay or immunoassay techniques which include membrane-based, solution-based, or chip-based technologies for the detection and/or quantification of the nucleic acid or protein.

Once a binding peptide conjugate is encoded the enzyme conjugate may be isolated and purified from the host cell by well-known techniques such as, cell separation and concentration of the cell free broth by ultrafiltration, ammonium sulfate fractionation, purification by gel filtration, ion exchange or hydrophobic interaction chromatography, PEG extraction and crystallization.

Methods of purification are well-known for many enzymes. One non-limiting example of purification of an enzyme conjugated to a binding peptide of the invention includes small-scale purification (e.g., less than 1 g) of the enzyme using hydrophobic interaction chromatography. Samples may be filtered and loaded onto a column containing 20HP2 resin (Perceptives Biosystems), hooked up to a BioCad workstation (Perceptives Biosystems). The column may be washed with ammonium sulfate in buffer. Elution of the derivatized phenol oxidizing enzyme activity can be performed using a salt gradient ranging from 35% to 0% of a 3M ammonium sulfate solution in 30mM Mes Bis Tris Propane buffer at pH 5.4. The fractions enriched in the derivatized phenol oxidizing enzyme activity can be monitored using UV absorbance at 280nm and a qualitative ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) activity assay. The samples can be pooled, concentrated and diafiltered against water. Enzyme samples purified according to this method are estimated to be at least about 70% pure.

G. Applications

The binding peptides and binding peptide conjugates according to the invention may be used in numerous applications which include but are not limited to enzyme and cleaning compositions, food industry applications and personal care applications. Some of these applications are discussed below, but the specific examples should not be interpreted as limiting any general application.

Enzyme and detergent compositions.

A binding peptide conjugate of the present invention may be used to produce, for example, enzymatic compositions for use in detergent or cleaning compositions; such as

Food industry applications.

Tannins are important taste components in tea and wine. In wine, tannins come from the skin and seed of red grapes and from the wooden oak barrels used in the fermentation and aging process. Tannins, which can bind to proteins in saliva, cause the proteins to precipitate and result in a stringent or bitter taste. Various tannins are found in wine during the early stages of the fermentation process. During the later stages of the fermentation process many of these tannins are extracted from the wine. A binding peptide or a binding peptide conjugate according to the invention may be particularly useful in this wine aging process. By targeting a tannin compound in the early stage of the wine fermentation process the astringency of tannins could be reduced or eliminated in the wine.

Personal care applications.

Tannins and anthocyanin compounds are natural dyes and may act as ultraviolet light protectants, tan enhancers and astringents. By either direct addition of tannin or anthocyanin binding peptides, which may displace tannins or anthocyanins from the compounds they bind, or by addition of a binding peptide conjugate one could modify the action of these compounds on various biological tissues particularly teeth, nails and skin. A non-limiting example includes a conjugated binding peptide comprising a peptide linked to a bleaching agent, wherein the conjugate delivers the bleaching agent to stained teeth for the purpose of bleaching. Another non-limiting example includes providing a binding peptide to the skin wherein astringency may be modified. Personal care products including a binding peptide may be formulated as creams, lotions, ointments and the like.

Having thus described the binding peptides and binding peptide conjugates of the present invention, the following examples are now presented for the purposes of illustration and are neither meant to be, nor should they be, read as being restrictive. Dilutions, quantities, etc. which are expressed herein in terms of percentages are, unless otherwise specified, percentages given in terms of per cent weight per volume (w/v). As used herein, dilutions, quantities, etc., which are expressed in terms of % (v/v), refer to percentage in terms of volume per volume. Temperatures referred to herein are given in degrees centigrade (C).

EXPERIMENTAL

Example 1: Selection of the binding peptides on tea and wine stained cotton.

While a number of selection techniques may be used to screen for binding peptides, the majority of the binding peptides according to the invention were selected according to the method described herein below.

10 microliters of a commercially (New England Biolabs) available phage display library either a cyclic 7-mer (at 2 x 10^3 pfu/ml) or a linear 12-mer (at 4 x10^{12} pfu/ml) were pre-incubated with a cotton swatch in a pre-blocked and washed 96 well plate in the presence of a 150 µl Tris-buffered saline (TBS) solution (at 2 x 10^{-5} g/l for the cyclic 7-mer, 2 x 10^{-3}g/l for the linear 12-mer) of detergent, pH 10 for 20 minutes using gentle shaking. The solution was pipetted off and added to a second cotton swatch for 20 minutes under gentle shaking. This process was repeated a third time. The solution was pipetted off and added to a tea or wine stained cotton swatch (Textile Innovators Corp. Windsor, NC) for 60 minutes under gentle agitation. The solution was drawn off and discarded. The stained swatch was washed 5 times for 5 minutes each with 200 µl of TBST (TBS containing 0.1% Tween 20). The swatch was transferred to an empty well using sterile tips, washed as described above, and transferred to another empty well. 15 µl of a glycine 0.2M solution pH 2.2 was added to the stained swatch and the plate was shaken for less than 10 minutes. This solution was neutralized by the addition of 100 µl of a Tris HCL 1M solution, pH 9.1 for 10 minutes. The solution, which constitutes the acid eluted peptide population, was pipetted off and stored at 4°C until further use.

Example 2: Amplification of the acid eluted peptides.

4 X 20 µl of the acid eluted phage peptide population was used to infect 4 X 400 µl E. coli (New England BioLabs) grown to an OD at 610 nm of 0.3 to 0.65 from a 100X dilution in LB of an overnight culture. The cells were plated on 4 X 140 mm LB plates in the presence of IPTG (Sigma) (40 µl at 20 mg/ml per plate) and Xgal (Sigma) (40 µl at 40 mg/ml of DMF per plate), added to 5 mls of melted top agarose, and left to incubate overnight at 37°C. The 4 plates were scraped with a sterile glass microscope slide and the scrapings were pushed through an 18.5 gage needle of a 60 ml syringe into a sterile conical tube; 50 ml of TBS was added to the tube and the capped tube was left to shake on a rocker at room temperature for at least 14 hrs. The contents of the tube were centrifuged at 10,000 rpm for 30 minutes in sterile Oakridge tubes at 4°C. The supernatant was collected and the phage precipitated by adding 1/6 volume of a 20% polyethylene glycol (PEG)/2.5 M NaCl solution. This was left to incubate at 4°C for at least 4 hours and preferably overnight. The solution was then spun at 10,000 rpm for 30 minutes at 4°C and

the supernatant discarded. The pellet was resuspended in 1 ml of TBS and transferred to a sterile Eppendorff tube. The phage was reprecipitated with 1/6 volume of a 20% PEG/ 2.5 M NaCl solution with incubation on ice for at least 1 hour. This was followed by another centrifugation at 10,000 rpm for 10 min at 4°C. The supernatant was discarded, the tube re-spun briefly, and residual supernatant removed. The pellet was resuspended in 200 µl TBS/0.02% NaN_3, spun to remove insoluble material and transferred.

Example 3: Biopanning.

The amplified phage peptide populations from the first round of deselection on cotton /selection of stained cotton swatches were submitted to another round of deselection and selection as described above. For the cyclic 7-mer peptide library 2 x 10^{-4} g/l TBS was used, and for the linear 12-mer peptide library 2 x10^{-2} g/l TBS was used. After acid elution and amplification of the phage, a third round of biopanning was performed. The third round used 2 x10^{-3} g/l TBS of detergent for the cyclic 7-mer phage peptides and 2 x 10^{-1} g/l TBS for the linear 12-mer phage peptides. After acid elution and amplification, a fourth round of biopanning was used and 2 g/l of detergent dissolved in water in one experiment and TBS in another were used for both types of phage peptides. The phage peptides were acid eluted and amplified from the fourth round of biopanning and selected in a fifth round of biopanning wherein the Tween 20 concentration was increased from 0.1% to 0.8% in the wash conditions. Additionally a round of selection on tea and wine was performed using the phage peptides from the third round as described above. In this fourth round of biopanning, 2 g/l of detergent in water in the wash conditions was used. One skilled in the art is well aware that various parameters as described hereinabove may be varied without affecting the nature of the invention. The above described method is one method which may be used to screen for binding peptides of the invention.

Example 4: Selection of the binding peptides on stained cotton after biopanning:

225 µl of a 1/100 dilution of an overnight culture of E. coli cells in LB broth were incubated with phage plaques using sterile toothpicks in a sterile 96-well V-bottom plate. A replica plate was made for glycerol stocks of the phage peptides. The plates were covered with porous Qiagen plate sealers and shaken for 4 hours at 37°C at 280 rpm in a humidified shaker box and then spun at 4000 rpm for 30 min at 4°C. 160 µl of the phage peptides supernatant was transferred to another 96-well V-bottom plate containing 64 µl of 20% PEG/2.5 M NaCl. The plates were left to shake for 5 minutes and then left to stand for 10 minutes. The glycerol stock plate was prepared by adding 100 µl phage supernatant to 150

µl 75% glycerol solution in a sterile 96 well plate which was then sealed with parafilm, labeled, and stored at –70°C until further use.

The PEG precipitated phage plate was centrifuged at 4000 rpm for 20 minutes at 4°C. The plate was inverted rapidly to remove excess PEG/ NaCl and left upside down on a clean paper towel to drain residual fluid. 60 µl of iodide salt solution (10 mM Tris.HCl, pH 8.0, 1 mM EDTA, 4 M NaI) were added to each well and the phage pellets thoroughly resuspended by shaking the plate vigorously for 5 minutes. 150 µl of 100% EtOH were added and the plate was spun at 4000 rpm for 20 minutes at 4°C, the supernatants discarded and the plate blotted. The pellets were washed with 225 µl of 70% EtOH without disturbing the pellets; the plate was inverted and left to air-dry for at least 30 minutes. The pellets were resuspended in 30 µl of Tris.HCl 10 mM, pH 8.5 buffer by shaking the plate for 30 minutes at full speed. 1 µl of g96 reverse primer (obtained from New England BioLabs, 3.4 pmole per tube) was added to 11 µl of DNA pellet sample and the contents submitted for sequencing on a ABI Applied Biosystem 373XL.

By raising the concentration of detergent in every round of biopanning and additionally during the washes; the stringency of the selection and wash steps was increased. In so doing, only those peptides that bind specifically to compounds in tea or wine remain bound after successive selection/wash steps in increasing detergent concentrations. Accordingly, increasing concentration of detergent between biopanning rounds, improves the number of phage that contains real peptide binders, and reduces the number of false-positives. Thus this approach helps improve the signal to noise ratio in this biopanning procedure.

Figures 1A - 1B and 3A - 3C (SEQ ID NOs.1 – 111 and 206 - 316) illustrate the amino acid sequences of numerous binding peptides determined according to the method described in examples 1 - 4.

Example 5: Selection of the phage binding peptides on tea stained ceramic

Deselection as described above was performed three times on unstained pieces of a ceramic teapot in a blocked 96 well plate using either cyclic 7-mer, linear 7-mer, and or linear 12-mer phage peptide libraries in the presence of a commercially available dish detergent. Selection was then performed on tea stained pieces of ceramic. The tea stained ceramic pieces were rinsed in TBST. After acid elution and neutralization, the tea stained ceramic pieces were further rinsed in TBS, dried and placed in PCR tubes. Lysis buffer was added to the tea stained ceramic and lysis was performed. The lysis solutions were subjected to a series of PCR reactions, TA cloning and sequencing as described above. The peptides were analyzed for the presence of repeatable motifs. Additionally, the PCR

products in the TA cloning step were amplified using PCR. The PCR fragments were digested with restriction enzymes and the resulting fragments were purified using standard phenol/chloroform extraction and ethanol precipitation procedures. The fragments were eluted on a 8% PA gel in TBE and fragments of interest were cut out with a razor blade and further purified using the Qiagen purification kit procedure. The purified fragments were ligated with vector and competent E. coli E2537 cells were transformed using well known techniques. The transformants were sequenced according to standard protocols and the corresponding phage peptide libraries amplified prior to a second round of selection and deselection.

Using amplified phage peptide libraries from the first round of selection, another round of deselection and selection was performed as described above. The tea stained ceramic pieces were rinsed with TBST prior to acid elution and neutralization. The pieces were then rinsed in TBS and dried. The phage peptide libraries bound to the tea stained ceramic were lyzed and their DNA amplified using a series of PCR reactions. TA cloning was preformed on the PCR products. The TA clones were picked for PCR and sequenced as described previously herein. The sequences were also analyzed for the presence of repeatable motifs.

Figures 2A - 2B (SEQ ID NOs. 112 - 201) illustrate the amino acid sequence of numerous binding peptides determined according to the method described in this example 5.

Example 6: Selective binding of phage bound peptides to tea stained cotton swatches.

The phages containing the peptides LHQNQKS (SEQ ID NO. 68), TNNTSPT (SEQ ID NO. 24), SWNTSPL (SEQ ID NO. 80), SYGPMTN (SEQ ID NO. 65), PNTTRHS (SEQ ID NO. 2), LWTSPQL (SEQ ID NO. 8), and WT phage (without a peptide insert) were amplified from the glycerol stocks described in example 4. The amplification procedure was done as described as in example 2. A drop of corresponding glycerol stock was added to 20 ml of LB broth containing 0.2 ml of an overnight culture of E. coli. The culture was left to grow at 37°C under vigorous shaking for 4.5 hrs, transferred to sterile 50 ml Oakridge tubes and centrifuged at 10 000 rpm for 10 min. The supernatants (17 mls) were added to fresh, sterile centrifuge tubes containing 3 ml of 20% PEG/ 2.5M NaCl solution (1/6 volume). The phages were precipitated at 4°C for at least 4 hr and then centrifuged for 15 min at 10,000 rpm at 4°C. The phage pellets were suspended in 1 ml of TBS, transferred to sterile 1.5 ml microfuge tubes and re-precipitated with 1/6 volume PEG/NaCl on ice for at least 1 hr. The tubes were again spun for 10 min at 4°C and supernatants discarded. The pellets were

suspended in 0.2 ml of TBS/0.02% NaN_3 solution and the solutions were spun for 1 min to remove any insoluble material. Supernatants were transferred to sterile screw cap tubes.

Small (1/8") swatches of tea stained cotton (Textile Innovators Corp. Windsor, NC) and unstained cotton were punched out (in duplicates for each phage peptide) and placed in a pre-blocked and washed multititer plate. 0.150 ml of a 10 X dilution of each titered phage peptide solution in detergent /TBS (0.001 g of detergent/L of TBS) was added to two tea stained swatches and to two unstained cotton swatches and left to incubate for 30 min at room temperature on a rocker, under mild agitation. Solution was pipetted off and the swatches were rinsed 9 times with 0.2 ml of a TBST solution. Swatches were transferred into fresh empty wells and rinsed another 9 times with 0.2 ml of a TBS solution. Each swatch was placed in a PCR tube. 0.1 ml of lysis buffer was added (10mM Tris.HCl, pH8.4, 0.1% Triton X100), and then subjected to lysis at 95°C for 10 min. 2 µl of a 100 X dilution in lysis buffer (of the contents of each PCR tube) were added to Light Cycler ™ (Roche) capillaries. 10 µl of the Light Cycler™ cocktail (Roche. Per tube: 5.3 ul water, 1.1 ul of $MgCl_2$, 1.2 µl of mix (ATP+ dye), 2.4 ul of primers) was added to the tubes and the contents briefly spun on a table top centrifuge. The capillary tubes were capped and run on the Light Cycler™ PCR instrument . The contents of the tubes were quantified against a series of dilutions of a known and quantified phage peptide standard. The fluorescent signal coming from the intercalating dye correlates to the amount of DNA (copies) (using melting point correction) and therefore number of phage peptide present.

Figure 4 shows selective binding of phage-bound peptides to tea stained cotton swatches as compared to non-stained cotton swatches. For each phage-bound peptide illustrated, the peptide binds to tea stained cotton at least 2 times greater than to non-stained cotton. The graph shows phage peptide sequences which contain repeatable motifs bind greater than WT. LWTSPQL (SEQ ID NO. 8) binds to tea stained cotton about 2.5 times more than WT binds to tea stained cotton. LWTSPQL (SEQ ID NO. 8) binds to tea stain about 5 times more than to non-stained cotton. PNTTRHS (SEQ ID NO. 2) binds to tea stain about 15 times more than non-stained cotton.